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STUDIES ON BLOOD VISCOSITY AT LOW SHEAR RATES

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ABSTRACT

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At shear rates ranging from 0.05 to 50 sec^{-1}, the logarithm of viscosity of heparinized and defibrinated blood obtained from man and dogs shows a linear relationship with the volume per cent of cells. Analyses of such semilog plots indicate that the dependence of plasma viscosity on shear rate can be attributed to the presence of fibrinogen and that the dependence of blood viscosity on volume per cent cells is unaltered by the removal of fibrinogen.

Dextran preparations obtained from two different sources have similar effects on blood viscosity which increases in proportion to the molecular weight and the concentration of the dextran used.

At a given shear rate and for a given cell percentage, the viscosity of heparinized blood obtained from five species of animals (elephant, man, dog, sheep, goat) shows a direct relationship to the mean corpuscular volume (MCV). Such correlation between viscosity and MCV is less marked in Ringer-washed cell suspensions prepared from the blood of these species.

Shrinkage of red cells by washing with a hypertonic solution results in an increase of mean corpuscular hemoglobin concentration (MCHC) and a rise of viscosity. Swelling of red cells by washing with a hypotonic solution causes a decrease of MCHC and a lowering of viscosity.

In endotoxin shock, the outward filtration of plasma fluid across capillary walls is accelerated and the concentration of macromolecules in this fluid is also increased.

STUDIES ON BLOOD VISCOSITY AT LOW SHEAR RATES

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INTRODUCTION

Since the viscosity of blood is strongly shear-rate dependent at low rates of shear approximating near-zero flow (1, 2, 3), it appears to be advantageous to examine the effects of physiological and pathological alterations in blood composition in this low shear rate range where the viscosity is high and likely to display the greatest changes. This has been done in the present investigations by utilizing a G.D.M. air-bearing couette viscometer (4) which, except in solutions of very low viscosity, permits readings to be made at shear rates down to 0.05 reciprocal seconds.

I. EFFECTS OF SHEAR RATE, RED CELL CONCENTRATION AND REMOVAL OF PLASMA PROTEINS.

The concentration of red cells has long been recognized as the major contributor to blood viscosity. This relation was examined through a wide range of hematocrit values and at various shear rates for heparinized and defibrinated human and dog blood, as well as for washed cells suspended in Ringer's solution. Regression analysis of the data indicates that for a given shear rate a linear relationship exists between the logaritim of the viscosity and the volume per cent of red cells (hematocrit value corrected for plasma trapping).

Figures 1 and 2 illustrate typical sets of data for heparinized human and dog blood respectively. The lines shown in Figure 3 represent the linear regression lines based on five such experiments on dogs. It is seen that the intercepts on the viscosity axis at zero cell concentration vary with the shear rate. The implication is that the viscosity of cell-free heparinized plasma is shear-rate dependent (non-Newtonian). Direct measurements on plasma gave supporting indication of this inference, although exact torque readings at the lowest shear rate (0.05 sec^{-1}) are too near the limits of sensitivity of the G.D.M. to be reliable. Nevertheless, the viscosity of heparinized plasma reveals a definite upward trend in the range of more dependable readings.

As shown in Figure 4, the linear relation between log of viscosity and volume per cent cells also holds for defibrinated blood, but the intercepts for all shear rates are nearly identical (within the standard error of estimate), indicating that serum is Newtonian. Direct measurements on serum down to the lowest shear rates compatible with the G.D.M. sensitivity also showed no dependence of viscosity on shear

rate. Included in Figure 4 are the corresponding plots for heparinized dog blood. The difference between the heparinized and the defibrinated samples at a given shear rate reflects the effect of fibrinogen on blood viscosity. Since the slopes of the defibrinated and heparinized curves are almost identical, it appears that although fibrinogen is the non-Newtonian element in the plasma, it does not effectively alter the dependence of viscosity on red cell concentration, at least for dog blood. The viscosity of defibrinated blood is always lower than that of heparinized blood at the same shear rate and red cell concentration. There are indications that the viscosities of defibrinated and heparinized blood may become equal at sufficiently high shear rates, but for the range considered in the present studies the viscosity of defibrinated blood is consistently less than that of the corresponding heparinized blood.

Figure 5 shows the regression lines for dog cells suspended in Ringer's solution. This pattern of curves is similar to those for defibrinated blood in that the intercept is again independent of shear rate within the standard error of estimate. The viscosity of the suspending medium is less than 1.0 centipoise as is indicated in the figure, and for any given set of conditions the viscosity of the Ringer suspension is less than either heparinized or defibrinated blood. Here again it may be noted that the slopes of the curves for any of the experimental shear rates are not significantly different for heparinized, defibrinated or Ringer suspensions, lending credence to the hypothesis that the mechanism of the viscosity dependence upon red cell concentration is determined primarily by the red cells themselves.

II. EFFECTS OF HIGH AND LOW MOLECULAR DEXTRANS

Since the preceding progress report the tests on the effects of high and low molecular weight dextrans on blood viscosity have been confirmed with the G.D.M. viscometer, and two series of dextran, one from Pharmachem (m.w. 16,200: 43,300; 84,000; 184,000) and the other from Pharmacia (m.w. 9,400; 35,700; 73,000; 153,000; 375,000), were studied.

The dextran was added to freshly-drawn heparinized dog blood by replacing a suitable portion of plasma containing sufficient dissolved dextran to give the desired concentration (usually 2%). This method of preparation avoids any changes in concentration of other constituents and permits strictly controlled tests. The actual concentration of dextran was determined by the anthrone method (5). The effect of elapsed time between preparation of samples and determination of viscosity was shown to be negligible by reversing the order of the determination in parallel experiments.

The results from both series of dextran were in essential agreement when corrections were made for slight changes in hematocrit which were present in experiments with the Pharmachem series. The red cells underwent slight shrinkage when the Pharmachem preparations, especially those with low molecular weights, were added because of the presence of sodium chloride in the dextran preparation. Pharmacia dextran, though less soluble, was free of these osmotically active impurities.

As shown in Figure 6, the 16,200 m.w. dextran in concentrations up to 3% has no detectable effect on blood viscosity at any of the shear rates down to 0.05 sec^{-1}, whereas the 84,000 m.w. dextran increases the viscosity markedly. The increase

of blood viscosity upon the addition of high molecular weight dextran cannot be ascribed to changes in plasma viscosity since these changes are negligible. The action is directly on the red cells, probably causing aggregation which would account for the associated increase in sedimentation rate as well as the reported deleterious effects of high molecular weight dextrans on flow through the microcirculation (6).

A report of the results of these investigations has been submitted for publication in the Journal of Biorheology. As a result of these studies, we now have an in vitro method of determining the specific effects of plasma expanders on fluidity or viscosity of blood. Additional experiments have been started to compare the effects of high and low molecular weight dextrans in vivo.

III. EFFECTS OF RED CELL SIZE ON VISCOSITY.

This problem has been approached in two ways:

A. Comparative studies on goat*, sheep*, dog, human and elephant† blood in which the mean corpuscular volumes (MCV) of the red cells are approximately 18, 40, 56, 90 and 120 μ^3 respectively.

B. Examination of the effects of osmotic alterations in the size of red cells suspended in hypo- and hypertonic Ringer's solution.

The actual MCV in each experiment was derived from the centrifuge hematocrit value corrected for plasma trapping. The latter was determined for the blood of each species by dilution with I^{131}-albumin to give the true cell percentage for comparison with the centrifuge hematocrit.

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* Made available to us by Dr. Gandal of the Bronx Zoo.

† Obtained through the generous help of Dr. Henderson and Mr. Schmidt of the Ringling Bros., Barnum and Bailey Circus while in New York.

A. COMPARATIVE STUDIES ON VARIOUS ANIMAL SPECIES.

Experiments were done on freshly-drawn heparinized blood (50 mg/100 ml). Additional small samples were allowed to clot to obtain serum for electrophoretic studies. Aliquots of the heparinized blood were placed in 5 or 6 test tubes and centrifuged. The desired ranges of cell concentration (from 10-15 up to 80-90 volume per cent) were prepared by transferring plasma from one tube to another and thus lowering or raising the cell percentage. An additional portion of the freshly-drawn heparinized blood was washed three times with isotonic Ringer's solution. The Ringer-washed cell suspension was also prepared into 5 or 6 samples with different volume per cent cells. After thorough mixing, a small amount of each heparinized blood sample and each Ringer-washed cell suspension was taken for measuring hematocrit and for determining the count and size distribution of erythrocytes with the Coulter Counter and Distribution Plotter. Viscosities were measured as quickly as possible after mixing at a temperature of 37° C and shear rates from 50 to 0.05 sec^{-1}.

In Figures 7, 8, 9 and 10, the logarithm of viscosity is plotted against volume per cent cells for the heparinized blood and the Ringer-washed cell suspension obtained from the five species studied. Because of the closeness of the viscosity values between dog and human blood and also between goat and sheep blood, the data on sheep and human blood are presented in Figures 9 and 10 respectively in order to avoid superimposition of too many curves. For the other three species (elephant, dog and goat), the semilog plots of viscosity against volume per cent cells are combined and shown in Figures 7 (Heparinized blood) and 8 (Ringer-washed cell suspensions). Thus Figures 7 and 8 serve to illustrate the species

differences in viscosity at various cell concentrations. In both types of samples, over a wide range of volume per cent cells, the viscosity for the elephant (MCV 120 μ^3) is higher than that for the dog (MCV 66 μ^3) which in turn is higher than that for the goat (MCV 18 μ^3). The data obtained from man (MCV 90 μ^3) agree rather closely with those from the dog, and the viscosity for the sheep (MCV 40 μ^3) is between those for the dog and the goat. These results indicate that the viscosities at corresponding shear rates and the same volume per cent cells fall in the order of red cell size. For example, at 0.05 sec^{-1} and 50 volume per cent the approximate viscosities for the heparinized blood obtained from elephant, man, dog, sheep and goat are 170, 100, 90, 30 and 20 centipoises respectively. These values are not altered significantly by subtracting the plasma viscosities of the individual species which are estimated to be from 2 to 6 centipoises at the shear rate of 0.05 sec^{-1}.

The data on Ringer-washed cell suspensions also indicate a correlation between cell size and viscosity, although the differences among the various species were less marked than in the case of the heparinized blood. For example, at a cell concentration of 50 volume per cent and a shear rate of 0.05 sec^{-1}, the viscosity values are: elephant 40, man 26, dog 26, sheep 15 and goat 10 centipoises.

The correlation between cell size and viscosity of Ringer-washed cell suspension provides more definitive evidence for the dependence of viscosity on cell size than such correlation found in heparinized blood. This is because in heparinized blood the suspending medium, i.e. the plasma, is not the same in the various species. Figure 11 shows the Analytrol scanning of serum proteins in the electrophoretic paper strips obtained from man, elephant, goat and dog. The protein

patterns are quite different. The gamma globulin fraction is lower in the elephant (5.3%) as compared with those in goat (15.7%), man (16.3%) and dog (15.7%). The alpha-1 fraction is higher in the goat (8.4%) as compared to elephant (5.2%), man (4.3%) and dog (2.1%). It is possible that the serum protein patterns in the various species are such that they exaggerate the species difference in viscosity in the same direction as the influence of MCV, and hence the correlation between viscosity and cell size is more striking in the heparinized blood than in the Ringer-washed cell suspension. It is interesting to note that the plasma viscosities of the five species studied do vary in the same direction as the MCV, e.g. at 0.05 sec^{-1} the values of plasma viscosity can be estimated as 3.1 centipoises for elephant, 2.8 for man, 2.2 for dog, 2.1 for sheep and 1.8 for goat.

For a given animal species, a comparison of the log viscosity-cell percentage relationship obtained from the heparinized blood with that obtained from the Ringer-washed cell suspension shows that the viscosity is higher in the heparinized blood which contains the plasma proteins. Such a comparison is shown for human blood in Figure 10, and similar differences are found for elephant, dog and goat blood. For the sheep blood (Figure 9), the viscosity of heparinized blood is higher than that of the Ringer-washed cell suspension only when the volume per cent cells is less than 30%.

As shown in Figures 7 (heparinized blood) and 8 (Ringer-washed cell suspension), the semilog plots of viscosity against volume per cent cells for the elephant yield straight lines as in dog and human blood. The corresponding data on the goat samples, however, do not fall on a straight line, curving upward as the cell percentage rises above 40-50%. Data on sheep blood (Figure 9) also indicate such curvature when the cell concentration is above 40-50%.

For the heparinized blood samples, shear stress was calculated (= product of shear rate and viscosity). The square root of shear stress is plotted against the square root of shear rate (Casson plot, 7) and the curve is extrapolated down to give the "yield stress" corresponding to zero shear rate. It is found that the yield stress (τ_y, Figure 12) shows significant species differences and appears to be related to the variations in MCV.

D. OSMOTIC ALTERATION OF WASHED RED CELLS.

Freshly-drawn heparinized human and dog blood was used. Aliquots of blood were washed in three kinds of salt solutions with different osmotic concentrations. All three solutions contained 0.042 gm% KCl, 0.024 gm% $CaCl_2$ and 0.02 gm% $NaHCO_3$, but different amounts of NaCl: 0.7 gm% (hypotonic), 0.9 gm% (isotonic) and 1.3 gm% (hypertonic). For each type of washed cell suspension, samples of different cell percentages were prepared. Preliminary tests have shown that for a given volume% red cells, the viscosity is increased by cell shrinkage in hypertonic solution and reduced by cell swelling in hypotonic solution. It should be pointed out that the variations in MCV in these instances are accompanied by opposite changes in the mean corpuscular hemoglobin concentration (MCHC). Thus the rise in viscosity found in hypertonic medium is associated with an increase in MCHC and the reduction of viscosity in hypotonic solution is associated with a decrease in MCHC. Such correlation between viscosity and MCHC is in accord with the relative viscosities recently reported by Erslev and Atwater (8), using a capillary viscometer in which the shear rate is not specifically known.

IV. DEVELOPMENTS IN INSTRUMENTATION

In the Bell Telephone Company Laboratories at Murray Hill, a new rotor and drive mechanism has been designed and built for the G.E.M. viscometer. With this advancement in instrumentation, we are able to control temperature within narrow limits and make precise shear rate settings through a continuous scale from 80 to 0.0002 sec^{-1}. This will eliminate two sources of error in making precise viscosity measurements. The Bell Telephone Laboratories are also exploring further new possibilities for increasing the sensitivity and precision of torque measurements in the extremely low range.

A Zimm viscometer (8) is being assembled and will be used for determining viscosities of plasma, serum and plasma protein solutions at low shear rates. This will enable us to obtain further information concerning the role of fibrinogen in the shear-rate dependence of plasma and blood viscosities.

V. STUDIES ON ENDOTOXIN SHOCK

This form of circulatory shock has been investigated partly with the view that it would be a suitable experimental model for exploration of viscosity factors in stagnant hypoxia and in the irreversible stage of shock.

In dogs under pentobarbital anesthesia, the thoracic duct was cannulated. Dextran with mean molecular weight of 250,000 (Dx) and I^{131}-albumin (RISA) were injected intravenously and their concentrations determined in the plasma (P) and the thoracic duct lymph (L) at 20-min. intervals for 100 min. E. coli endotoxin (3 mg/kg i.v.) was then administered. As the arterial pressure decreased, the lymph flow from the thoracic duct increased within 5 min. after endotoxin and reached a peak (approximately double the control) in 10 min. At the same time

$(Dx)_L$ and $(MBA)_L$ increased markedly. Since $(Dx)_P$ and $(MBA)_P$ usually decreased slightly, the L/P ratio for these macromolecules increased. Eighty min. after endotoxin injection, the L/P ratios remained high (0.65 for Dx and 0.85 for MBA) although the lymph flow already declined to within 10% of the control. In control dogs not given endotoxin, the L/P ratio was only 0.35 for Dx and 0.55 for MBA at 180 min. after the injection of these macromolecules.

The results of a typical endotoxin experiment are shown in Figure 13. The mean values and the standard errors of mean from 8 such experiments are compiled and plotted in Figure 14. The shaded areas in Figure 14 indicate the results obtained from control dogs which did not receive endotoxin. The data indicate that endotoxin caused a marked increase of the outward passage of fluids rich in macromolecules across the capillary membrane of the splanchnic area. Since the increase in lymph flow after endotoxin was associated with rises in portal and wedged hepatic venous pressures and decreases in central venous pressure and hepatic blood flow, the outward movement of fluid can be explained by an increase of hydrostatic pressure in splanchnic capillaries due to hepatic venular constriction.

ACKNOWLEDGEMENTS: The investigations covered in this report were carried out with the collaboration of Drs. Chu Chien, Chen Chang, Robert Dollenback, Duncan Sinclair and Mr. Harry Taylor.

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A LIST OF KEY WORDS

Dextran

Endotoxin shock

Viscosity, blood

Viscosity, blood, species difference

Viscosity, plasma

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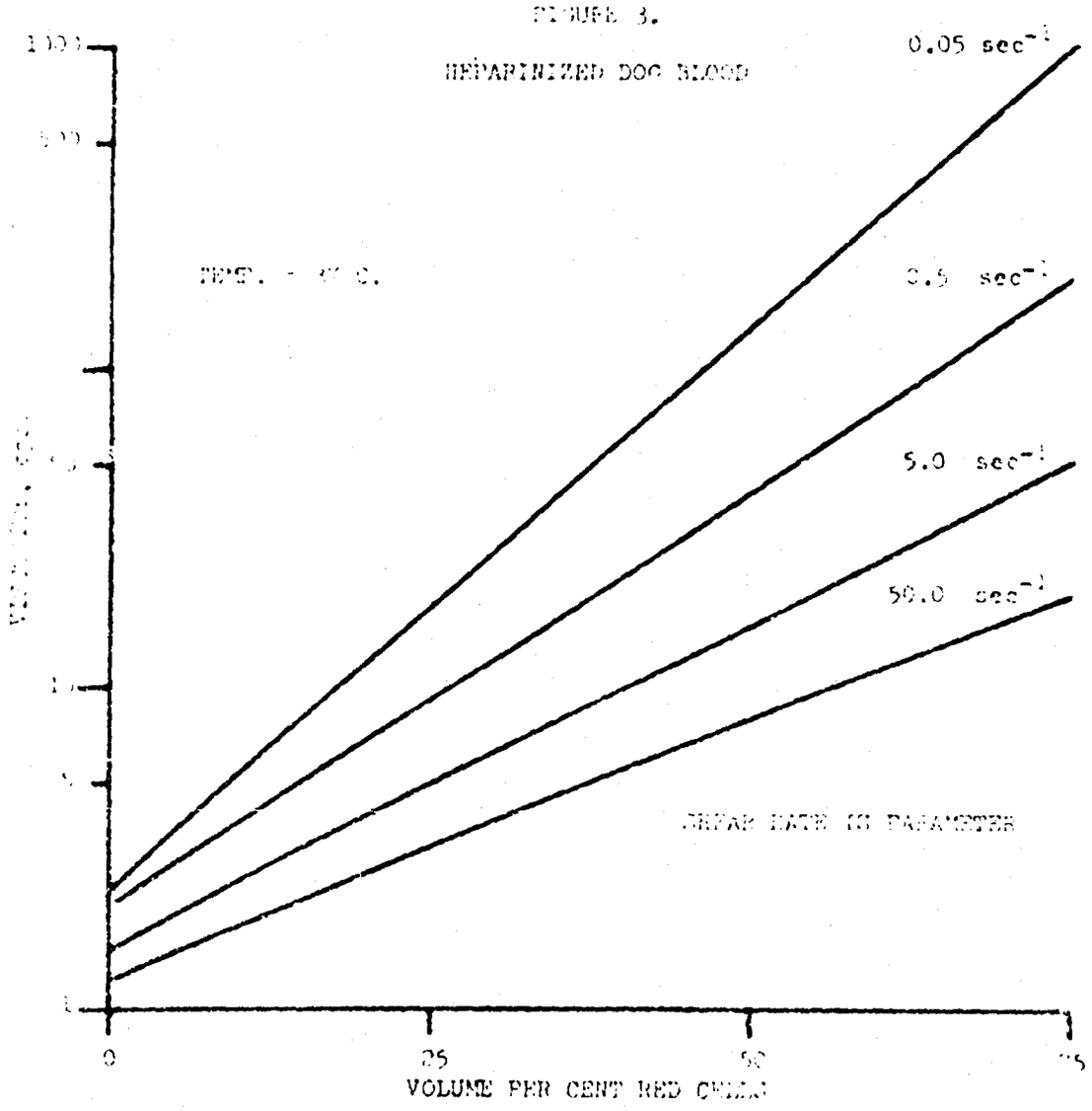
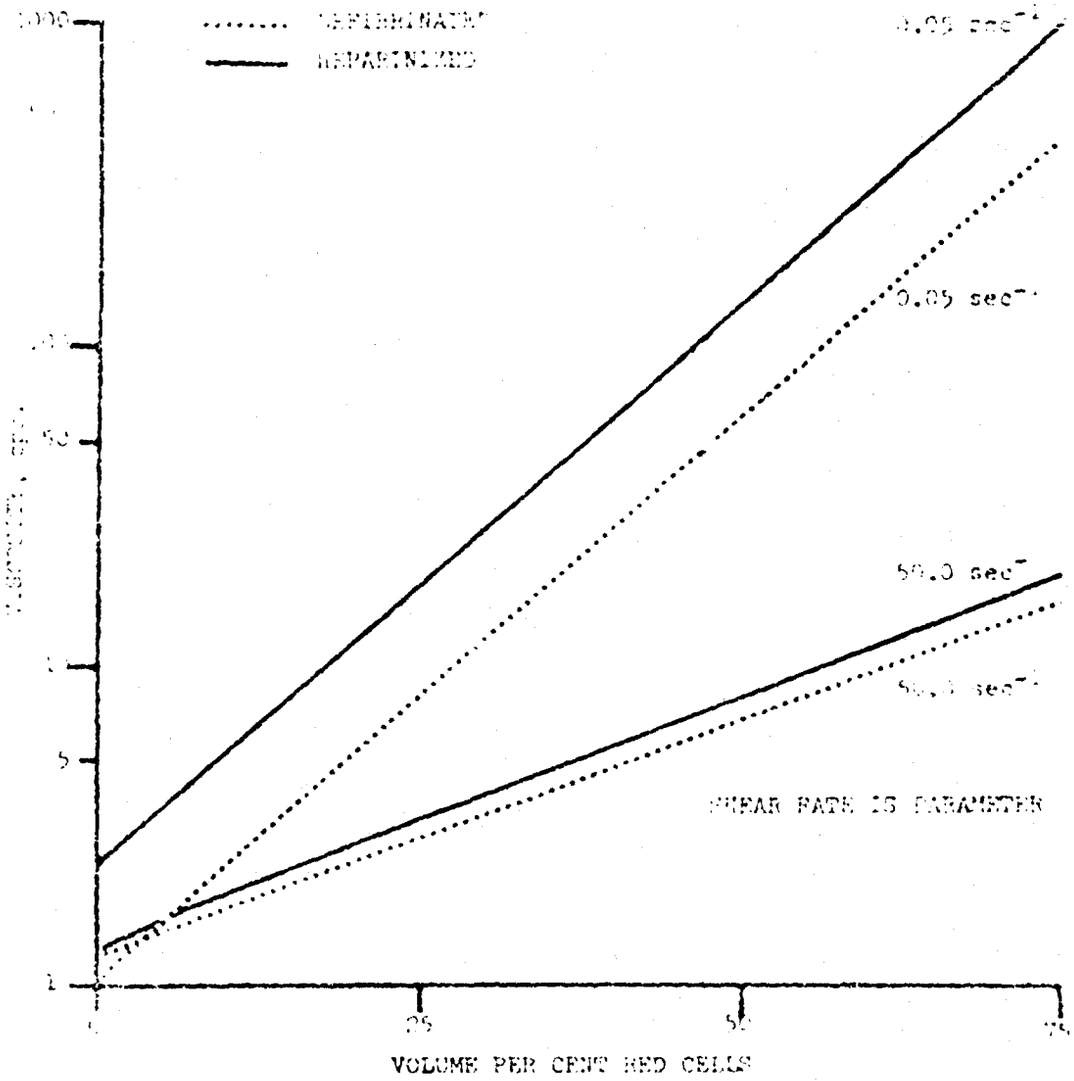


FIGURE 3.

HEPARINIZED DOG BLOOD

FIGURE 4.

COMPARISON OF HEPARINIZED AND DEFIBRINATED DOG BLOOD



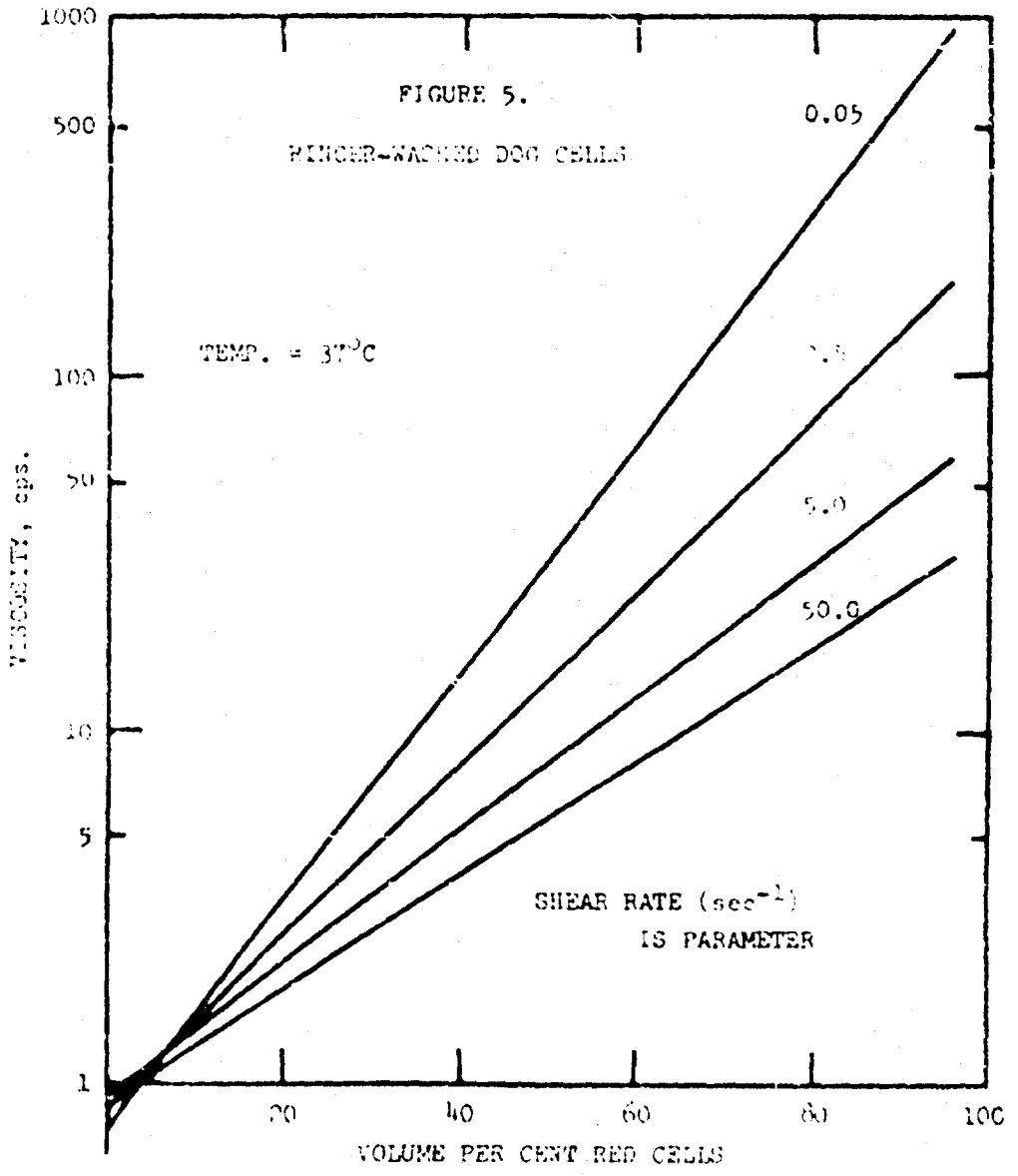
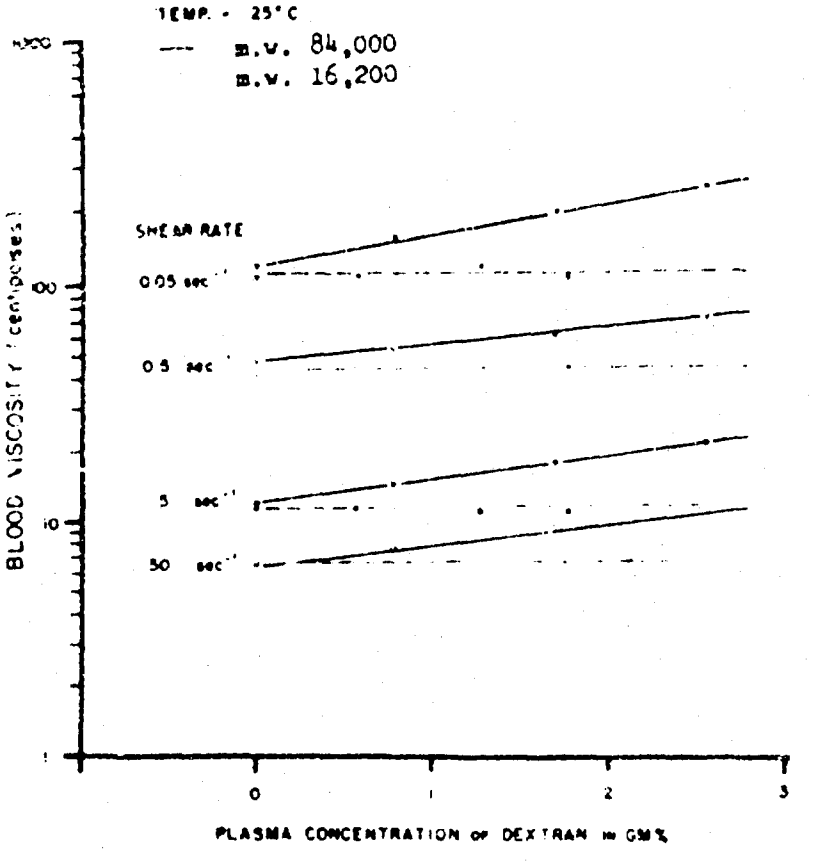
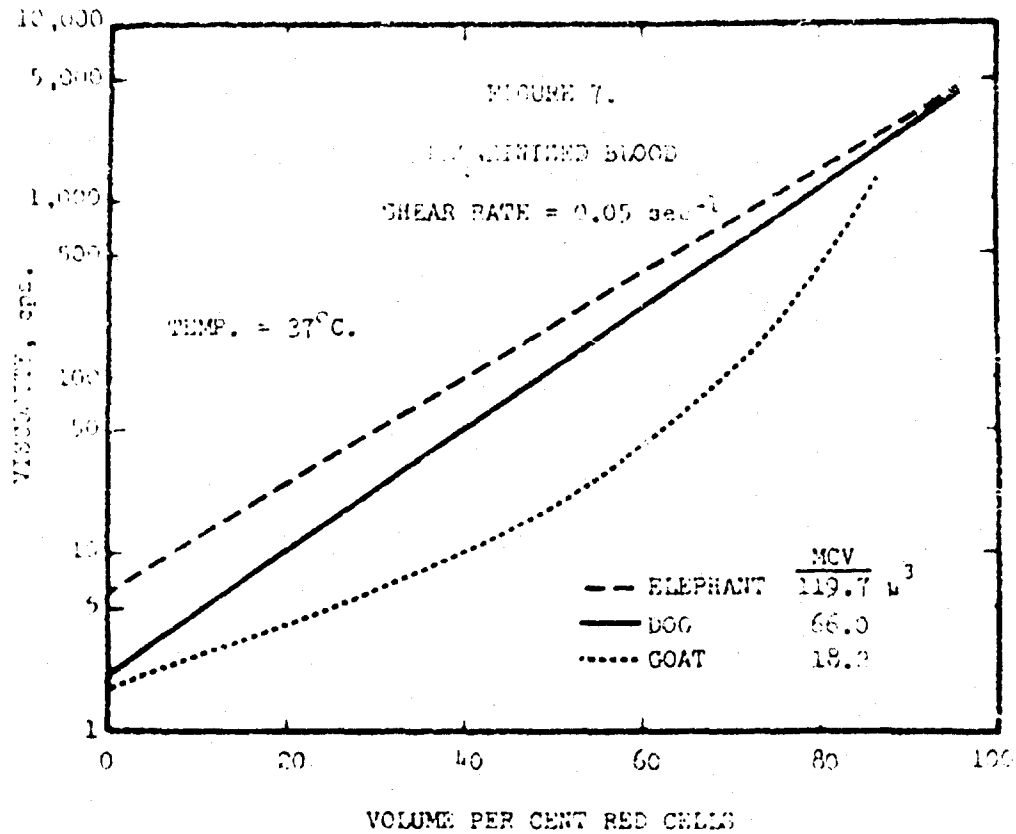
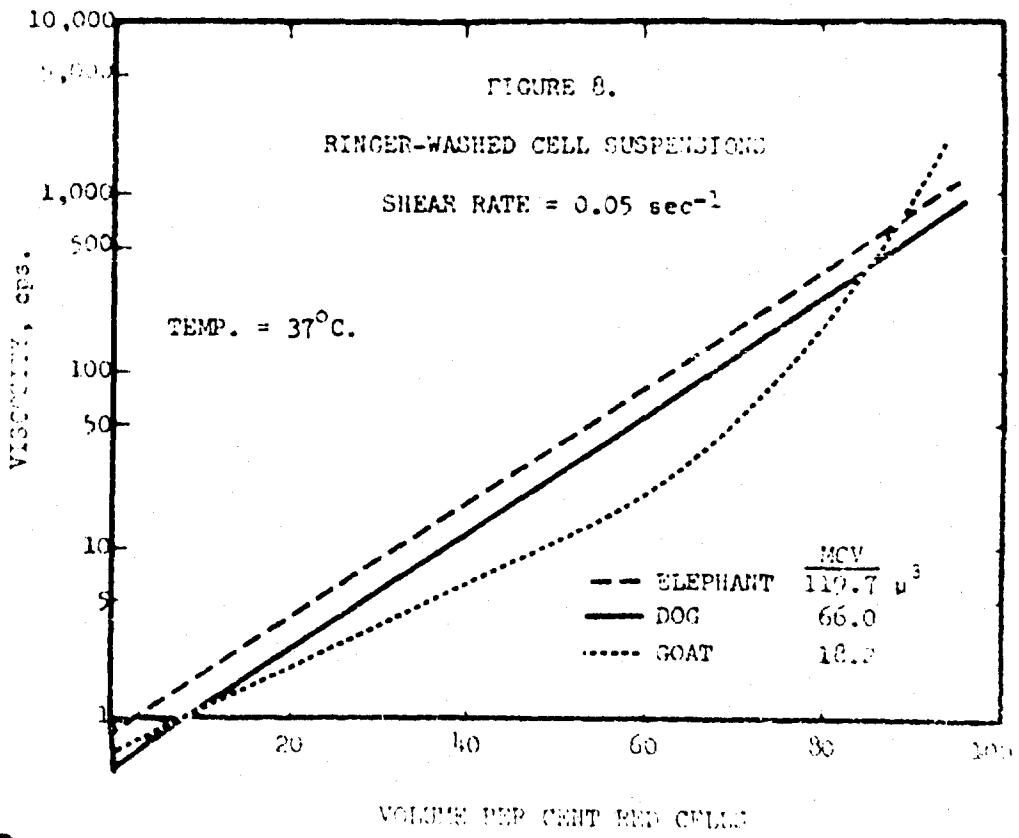


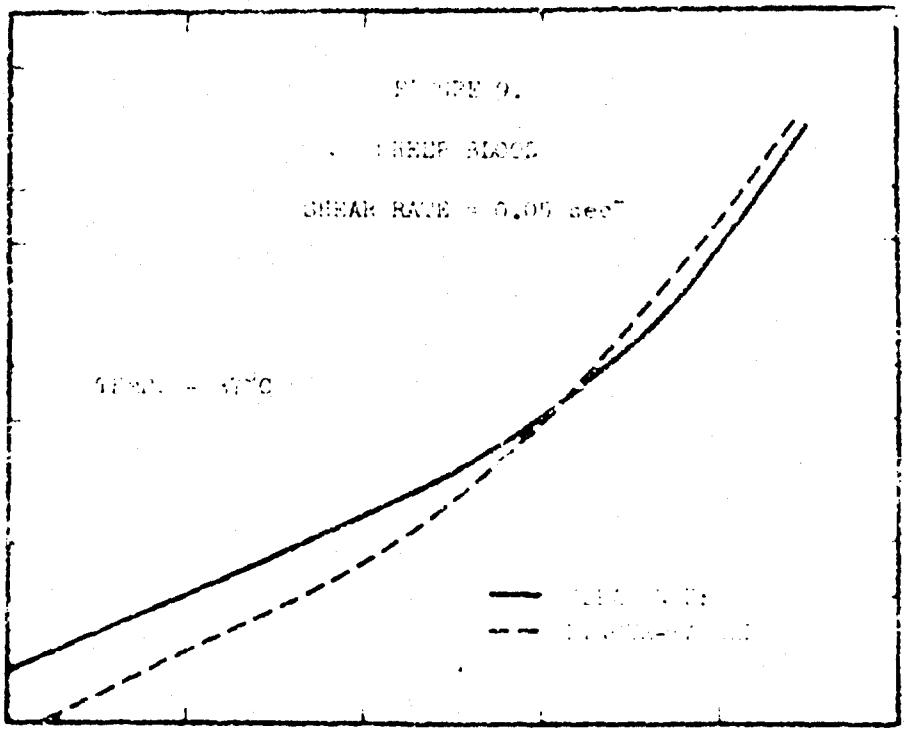
FIGURE 6.

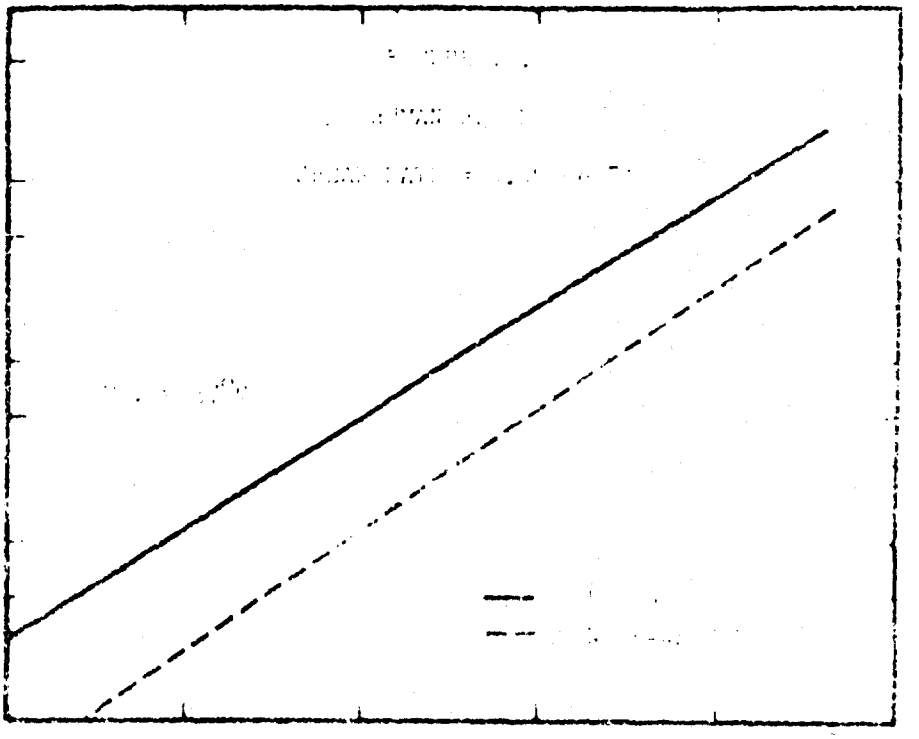
EFFECT OF DEXTRAN

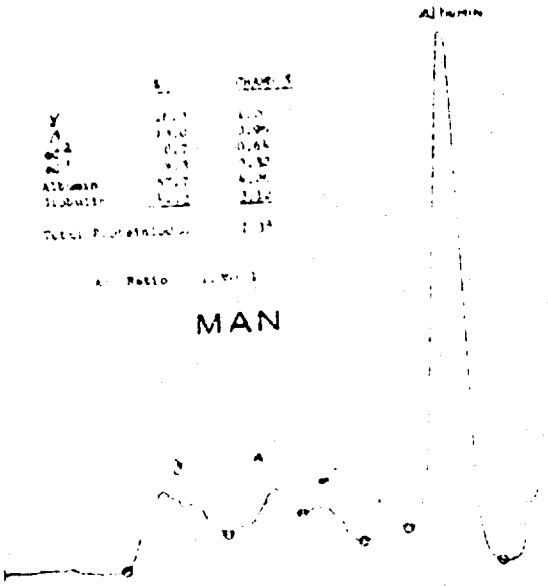


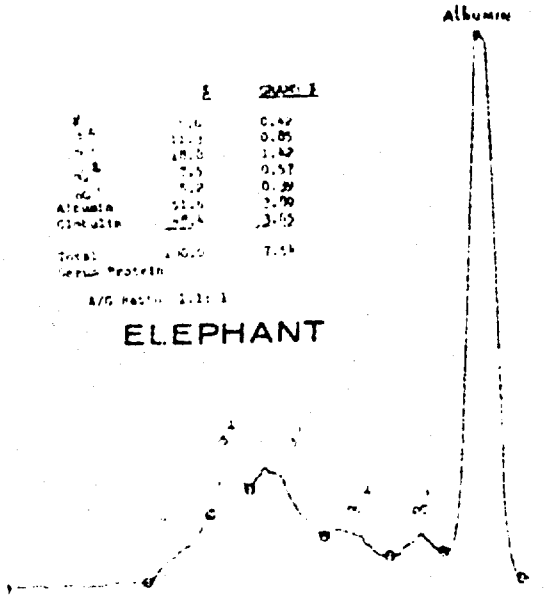


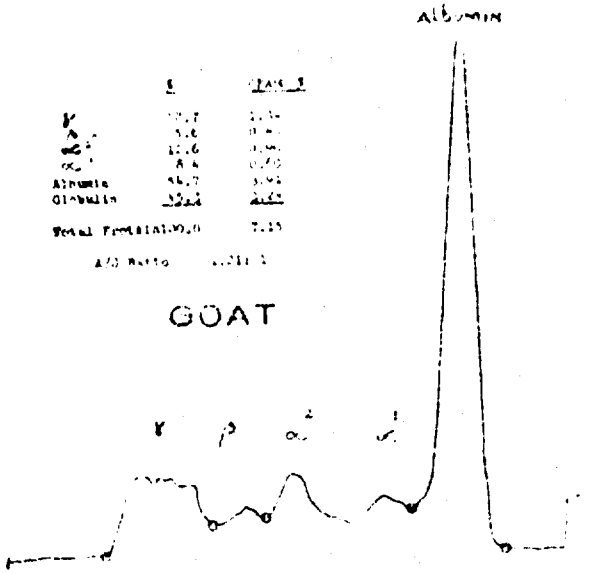


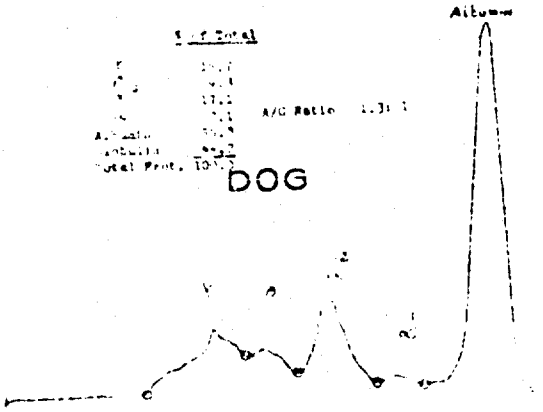












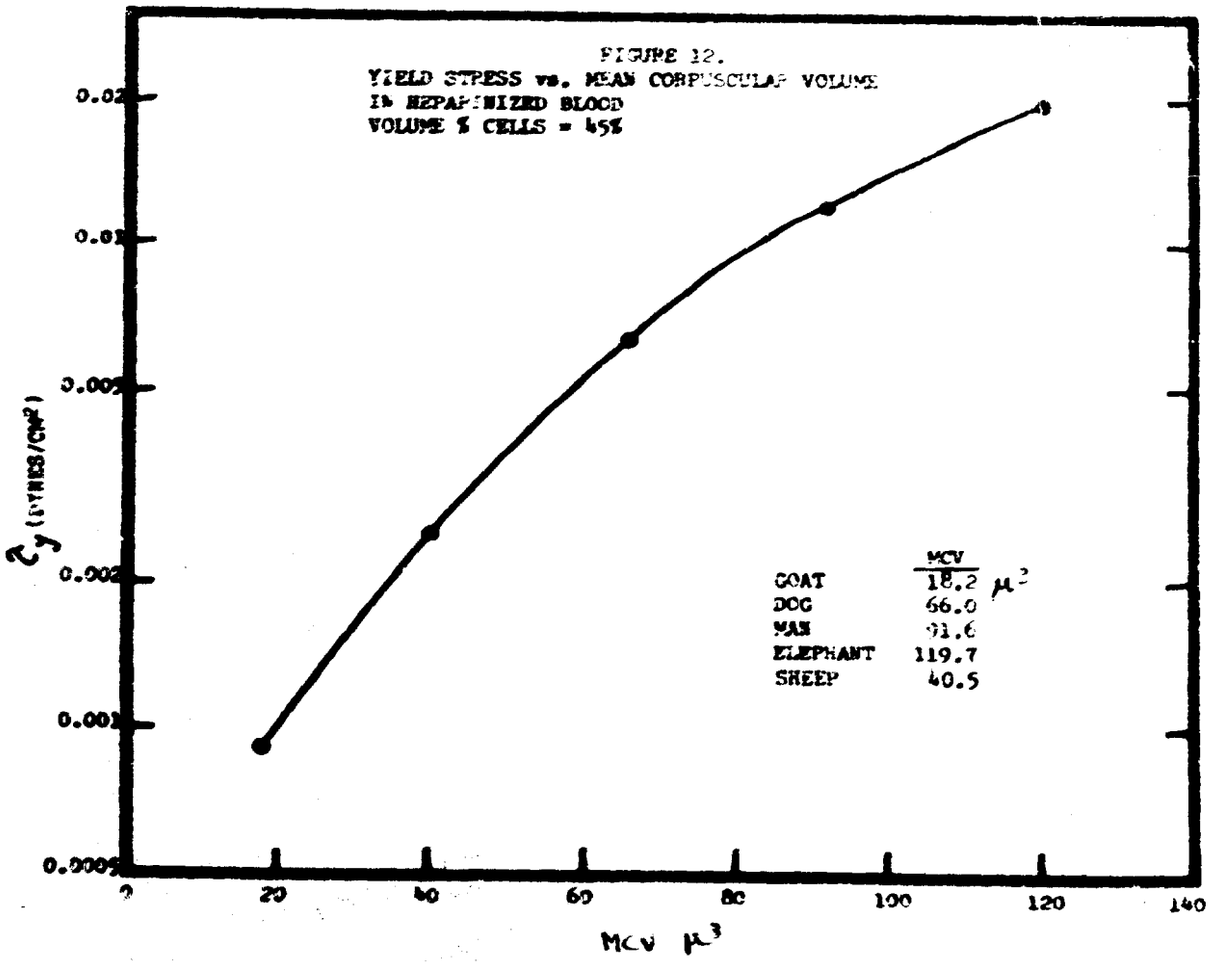


FIGURE 12.
YIELD STRESS vs. MEAN CORPUSCULAR VOLUME
IN HEPARINIZED BLOOD
VOLUME % CELLS = 45%
0.02
0.01
0.002
0.002
0.001
0.0005
τ_y (DYNES/CM²)
0
20
40
60
80
100
120
140
MCV μ^3
MCV
GOAT 18.2 μ^3
DOG 66.0
MAN 91.6
ELEPHANT 119.7
SHEEP 40.5

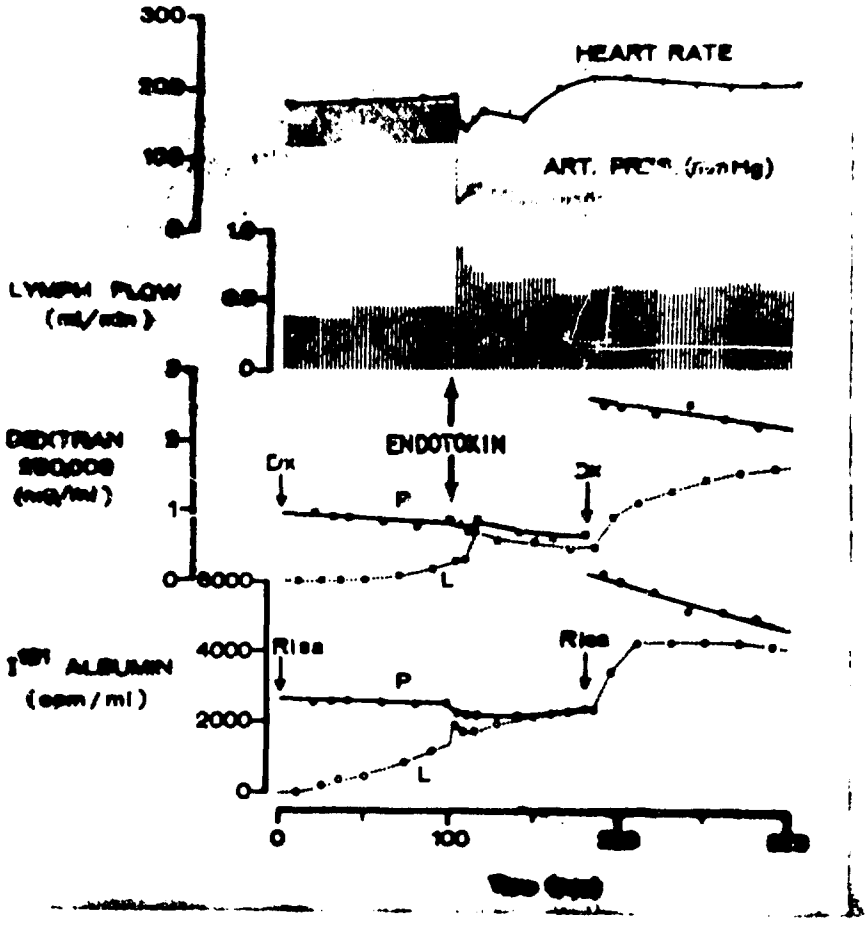


Fig. 13

Fig. 14